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Deparia cataracticola (Woodsiaceae), a New Species from Hawaii

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A new Hawaiian fern, *Deparia cataracticola* (Woodsiaceae), is described. It is morphologically distinct and ecologically unique among the Hawaiian species of *Deparia*. A molecular phylogenetic analysis showed that *D. cataracticola* is sister to a clade of *D. marginalis* and *D. prolifera*.

Key words: *Deparia cataracticola*, fern, Hawaii, molecular phylogeny, taxonomy

The genus *Deparia* consists of four sections, *Athyriopsis*, *Dryoathyrium*, *Lunathyrium*, and *Deparia* (Kato 1977, 1984). The former three sections had been treated as independent genera (Ching 1964, 1978; Chu 1999) or sections of the genus *Lunathyrium* (Ohba 1965). Kato (1984) studied the taxonomy of *Deparia* of the Pacific and adjacent regions. Section *Athyriopsis* in those regions includes 13 species; *Dryoathyrium*, 6 spp.; *Lunathyrium*, 4 spp.; and *Deparia*, 5 spp. Many additional species occur in continental Asia and Africa (including Madagascar), whereas only one species occurs in North America and no species is known from Europe and South America. Recently Chu (1999) revised the Chinese species (14 spp. of *Dryoathyrium*, 19 spp. of *Lunathyrium*, and 15 spp. of *Athyriopsis*). In their molecular phylogenetic and morphological analyses, Sano *et al.* (2000a, b, c) showed that *Diplazium subsinuatum*, *D. tomitaroanum*, and *Dictyodroma* should be placed in *Deparia* and Kato's (1984) section classification should be revised.

On the Hawaiian Islands there are four endemic species of sect. *Deparia*, *D. fenzliana*, *D. prolifera*, *D. marginalis*, and *D. kaalaana*. *Deparia petersenii* of sect. *Athyriopsis* is naturalized. The fifth non-Hawaiian species of sect. *Deparia*, *D. bonincola*, is endemic to the Bonin and Volcano Islands (Kato 1984). In this paper I describe a new species of sect. *Deparia* based on plants growing in waterfalls on Kauai Island, the westernmost large island of the Hawaiian archipelago. I also utilized molecular methods to determine whether the plants might represent an interspecific hybrid or an ecotype of a variable species, which was inferred from an examination of herbarium specimens and field observations, and if not, to determine the systematic relationship of the species.

Materials and Methods

Species examined in the molecular phylogenetic analysis were all species of sect. *Deparia* except *D. kaalaana*, for which material was not available, and some species of sect. *Athyriopsis* and sect. *Dryoathyrium* (Table 1). I used

TABLE 1. Material of *Deparia* species used in the phylogenetic analysis.

Species (Section)*	Source	GenBank Accession No.		
		rbcL	*rbcL-atpB*	*trnL-trnF*
D. bonincola (Nakai) M. Kato (DE)	Japan: Bonin Is.; *Kato s.n.* (TI)	D43899#	AB046959	AB046970
D. cataracticola M. Kato & al. (DE)	Hawaii: Kauai I.; *Kato H-105* (TI)	AB046982	AB046968	AB046980
D. conilii (Franch. & Sav.) M. Kato (AT)	Japan: Yakushima I.; *Kato s.n.* (TI)	D43901#	AB046960	AB046971
D. fenzliana (Luerss.) M. Kato (DE)	Hawaii: Maui I.; *Hasebe s.n.* (TI)	D43900#	AB046961	AB046972
D. marginalis (Hilleb.) M. Kato (DE)	Hawaii: Kauai I., *Kato H-21* (TI)	AB046981	AB046962	AB046973
D. minamitanii Serizawa (AT)	Japan: Miyazaki; *Kato s.n.* (TI)	AB021717#	AB046963	AB046974
D. okuboana (Makino) M. Kato (DR)	China: Dali, Yunnan; *Kato et al. 1043* (TI)	D43903#	AB046964	AB046975
D. otomasui (Kurata) Serizawa (AT)	Japan: Kumamoto; *Sano 28* (CBM)	D43904#	AB046965	AB046976
D. petersenii (Kunze) M. Kato (AT)	Hawaii: Oahu I.; *Kato H-5* (TI)	D43905#	AB046966	AB046977
D. prolifera (Kaulf.) Hook. & Grev. (DE)	Hawaii: Oahu I.; *Kato H-65* (TI)	D43906#	AB046967	AB046978
D. lancea (Thunb.) Fraser-Jenkins (DE + AT)	Japan: Yakushima I.; *Sano 30* (CBM)	D43913#	AB046969	AB046979

* Classification follows Kato (1977, 1984) and Sano *et al.* (2000b). AT, sect. *Athyriopsis*; DE, sect. *Deparia*; DR, sect. *Dryoathyrium*.
# Sano *et al.* (2000b).

published *rbcL* sequence data of all species examined except of *D. cataracticola* and *D. marginalis*, which were sequenced in this study. Vouchers are deposited in the herbarium, Botanical Gardens, University of Tokyo (TI).

Leaf material was collected from plants that had been transplanted to the Botanical Gardens, Graduate School of Science, University of Tokyo. The leaves were frozen with liquid nitrogen and pulverized to a fine powder. Total DNA was isolated from the resultant pellet using the CTAB method of Hasebe & Iwatsuki (1990). Double-stranded DNA of the chloroplast gene *rbcL*, *trnL-F* spacer, and *atpB-rbcL* intergenic region was amplified by 35 cycles of symmetric polymerase chain reaction (PCR). Primers used were aF (= 1-1), TWNP1, cF (= N2-1), aR (= 2R), TW2PR, cR (= NN3-2) (Hasebe *et al.* 1994; Murakami *et al.* 1999) for *rbcL*; e and f (Taberlet *et al.* 1991) for *trnL-F* spacer; and GCTTTAGTCTCTGTTTGTGGTGACAT [a reverse sequence of 1-1 of Hasebe & Iwatsuki (1990)] and GTTGTACTTCACAAGTAACATT (produced from the *atpB-rbcL* region in *Oryza sativa*, *Nicotiana tabacum*, *Marchantia polymorpha*, and *Angiopteris lygodiifolia*) for the *atpB-rbcL* intergenic region. PCR procedures in the first cycle consisted of 2 min at 94°C for denaturation, 1 min at 45°C for primer annealing, and 2 min at 72°C for primer extension. Denaturation time at 94°C was reduced to 1 min during the next 33, 36 or 38 cycles. The extension time at 72°C was increased to 10 min in the last cycle. PCR products were purified by electrophoresis in 1.0% agarose gel using 1x TAE buffer. The gel was stained with ethidium bromide and the DNA was eluted using Geneclean II (Bio 101, CA). Purified PCR products were sequenced in both directions using a Taq dye deoxy terminator cycle sequencing kit (Perkin Elmer, CA) on an Applied Biosystems Model 377 automated sequencer (ABI, CA). Primers for sequencing were the same as those for PCR.

A total of 2247 bp nucleotides, i.e., 1206 bp of the *rbcL* gene, from positions 73 to 1278

(from the start codon in *Nicotiana tabacum rbcL*; Shinozaki *et al.* 1986), 766 bp of *atpB-rbcL* intergenic region, and 275 bp of *trnL-F* spacer, were used for each taxon in the phylogenetic analyses. Alignment was made using CLUSTAL W (Thompson *et al.* 1994). *Deparia okuboana* was chosen as the outgroup for the species of *Deparia* examined. In an all-fern tree obtained from a 1152 bp *rbcL* sequence analysis, the species was placed in the basal clade that was sister, with 100% bootstrap value, to the clade in which the species of *Deparia* under investigation were included (see Hasebe *et al.* 1995; Sano *et al.* 2000a). The phylogenies of the variable nucleotide sites of the *rbcL* gene, *trnL-F* spacer, and *atpB-rbcL* intergenic region were analyzed by the distance matrix method and the maximum parsimony method.

In an analysis using the distance method, phylogenetic trees were computed using the DNDIST, NEIGHBOR, SEQBOOT, and CONSENSE programs in the PHYLIP software package (Felsenstein 1995). The evolutionary distances were calculated using the DNDIST program and Kimura's (1980) two-parameter method. A gene tree was constructed with the neighbor-joining (NJ) method (Saitou & Nei 1987) as implemented by the NEIGHBOR program. The relative support of the resulting branches was estimated by a bootstrap analysis with 1000 replicates using the SEQBOOT and CONSENSE programs.

The most parsimonious trees were constructed from the aligned data using PAUP version 3.1.1 (Swofford 1993). I used heuristic search with 1000 random addition sequence option, based on equal weighting together with the Tree Bisection Reconnection (TBR) branch-swapping algorithm and MULPARS on, and Steepest Descent on. A bootstrap analysis was conducted with 1000 replicates to assess the internal support for clades found in the analysis.

Results and Discussion

Description of the new species

Deparia cataracticola M. Kato, **sp. nov.** (Fig. 1)

Ab congeneribus habitatione diversa, et ab *Deparia fenzlianae* laminis basi vix vel dilute decrescentibus, pinnis semipinnatifidis, soris medianis similis sed squamis fuscis vel atrofuscis, pinnis plerumque gemmiparis differt, ab *D. prolifera* soris medianis, ab *D. marginali* laminis dilute attenuatis differt.

Type. HAWAII, Kauai Island: Hanakapiai waterfall, Na Pali-Kona Forest Reserve, on wet, sprayed, mossy waterfall cliff, 300m alt., October 25, 1997, *M. Kato H-105* (holotype BISH; isotype TI, TNS).

Rhizomes ascending to erect with leaves fascicled. Petioles (8-)15-27 cm long, scaly at base, sparsely or hardly scaly upwards, ± hairy, or old leaves not hairy, stramineous; scales linear-lanceolate, acuminate, ± crisped, linear, to 10 mm long, 1-2 mm wide, brown or dark brown, entire; hairs multicellular. Laminae oblong-lanceolate, acuminate, hardly narrowed at base or with a few shortened basal pinnae, 25-40 cm long, 10-19 cm wide, herbaceous to chartaceous, bipinnatifid, rachis hairy, hairs multicellular, sparsely scaly, scales minute; middle pinnae linear, ± falcate, apex acuminate, subcordate or truncate, sessile, 7-13 cm long, 0.9-1.7 cm wide, lobed halfway to costa or a little more deeply; lobes of pinnae oblique, 3-5 mm wide, oblong, apex round, almost entire to serrate with a few weak teeth; veins pinnate, lateral veinlets 3-5 pairs, simple; costae and veins on lower surface hairy, upper surface glabrous; rachis and costae grooved on adaxially, grooves of costae not open to rachis groove, interrupted by ridges along rachis groove; gemmae (or plantlets on older fronds) abundant on upper surface of rachis and costae. Sori median from near midvein to near margin, linear,

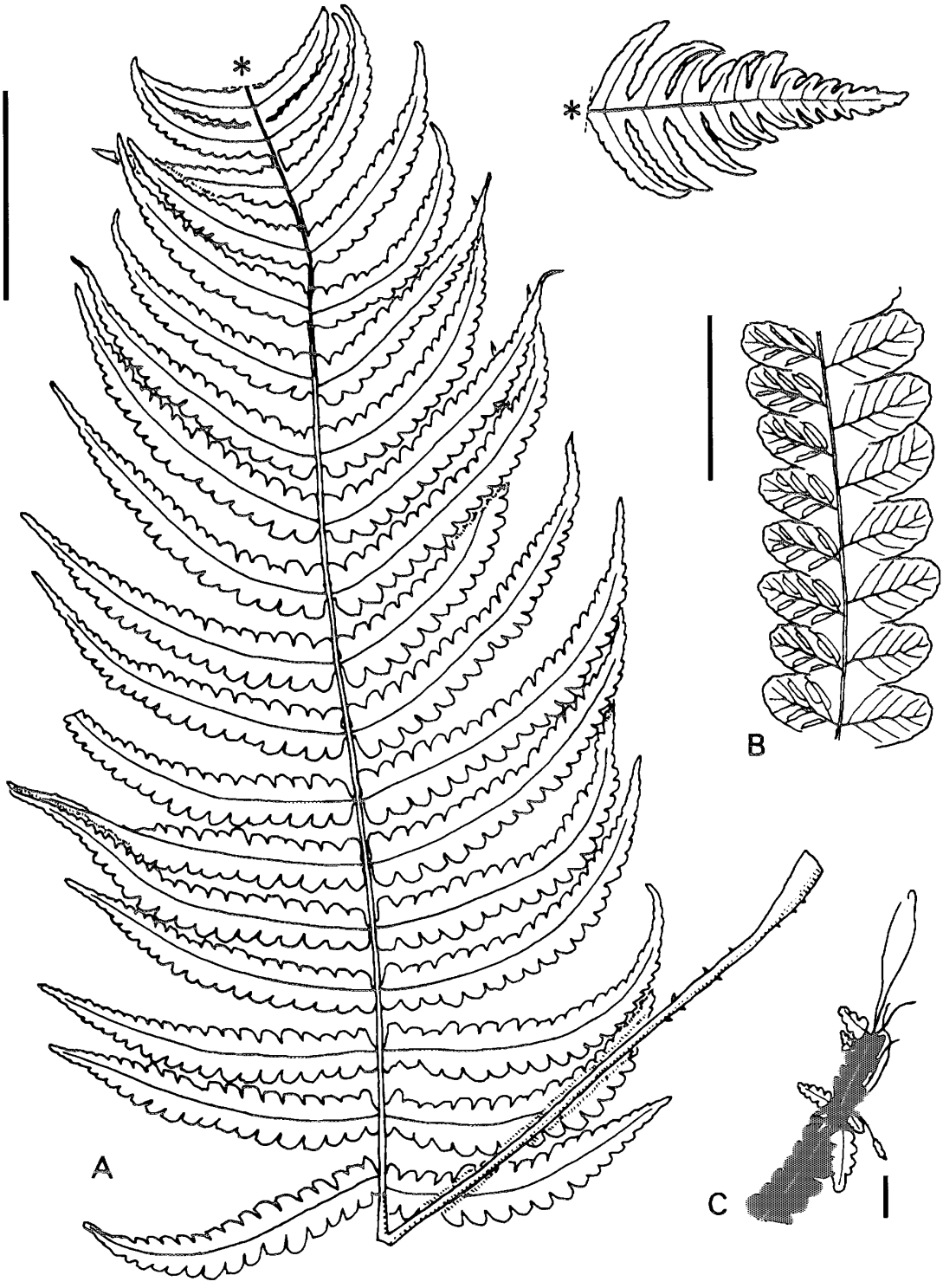

FIG. 1. *Deparia cataracticola*. A. Leaf (*Kato H-105*). Scale bar = 5 cm. B. Part of pinna (*Kato H-104*). Sori are illustrated on one side of pinna. Scale bar = 1 cm. C. Distal part of pinna (black) with plantlets on adaxial surface (*Kato H-105*). Scale bar = 1 cm.

single, or double along basal acroscopic veins, to 3(-4) mm long; indusia entire. Mature spores not seen.

Other specimens examined. HAWAII, Kauai Island: Hanakapiai waterfall, Na Pali-Kona Forest Reserve, on wet, sprayed, mossy

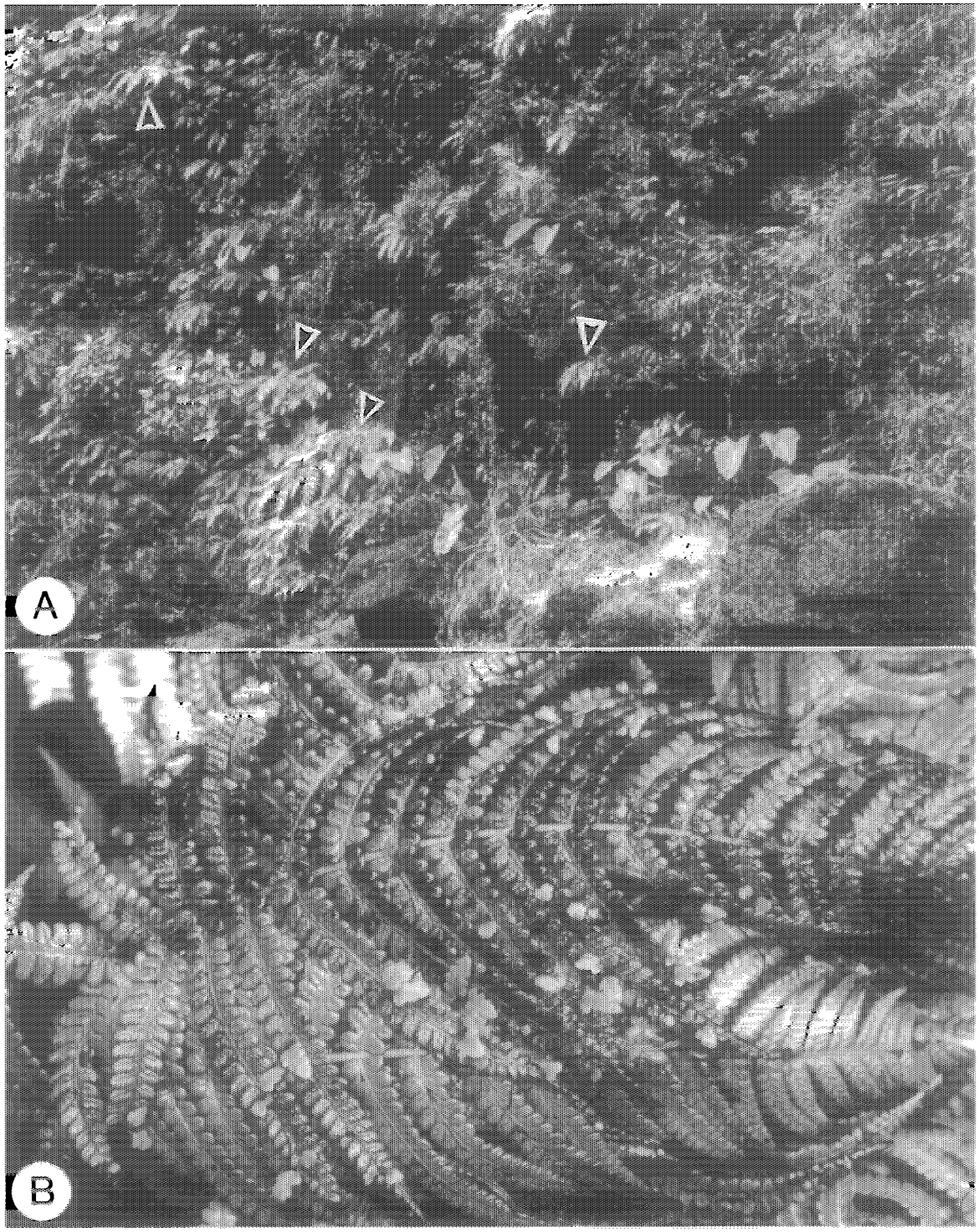

FIG. 2. *Deparia cataracticola* at Hanakapiai waterfall, Kauai. A. Wet sprayed cliff. Arrow indicates *D. cataracticola*. B. Plantlet-bearing old leaves.

waterfall cliff, 300m alt., October 25, 1997, *M. Kato H-104* (TI, TNS); Kauai Island, Northfork Wailua River, Lihue Koloa Forest Reserve, on wet steep waterfall slope at 520m alt., October 28, 1997, *M. Kato H-116* (TI, TNS).

This species of wet, sprayed, mossy cliffs in waterfalls is ecologically unique[#] (Fig. 2A).

Another ecologically unique species in *Deparia* is *D. kaalaana*, which is probably a rheophyte; most other species of *Deparia* occur in forests (Kato 1984).

Deparia cataracticola is also morphologically characteristic in having abundant gemmae or plantlets on the leaves, particularly on old leaves, as in *Woodwardia prolifera* and *Asplenium bulbiferum* (Figs. 1C, 2B). It is similar to *D. fenzliana* among the Hawaiian species of *Deparia* in the moderately reduced few basal pinnae, the pinnae lobed halfway to the costa, and the sori median from near the midvein to near the margin. It differs, however, in the brown or dark brown scales at the petiole base and in bearing gemmae on the upper surface of the pinnae. *Deparia cataracticola* differs from the other indigenous Hawaiian species of *Deparia*. The variable *D. prolifera* has the rhizomes creeping, a few to a moderate number of gemmae borne at the distal part of rachis and costae, the laminae up to tripinnatifid, and the sori submarginal to marginal. *Deparia marginalis* has the lamina gradually narrowed toward the base and only a few gemmae on the distal part of the rachis and costae. *Deparia kaalaana* has small leaves (petiole to 9 cm long, lamina to 26 cm long), the pinnae and pinnalobes distinctly oblique (characteristic of rheophytes), and a few gemmae on the distal part of the rachis. *Deparia cataracticola* also differs from the naturalized *D. petersenii* var. *petersenii*, which has long creeping rhizomes and toothed indusia. The following key will aid in identifying the Hawaiian species, indigenous and naturalized, of *Deparia*.

Key to the Hawaiian Species of *Deparia*

1. Rhizomes ascending to erect or short-creeping; margin of indusia entire

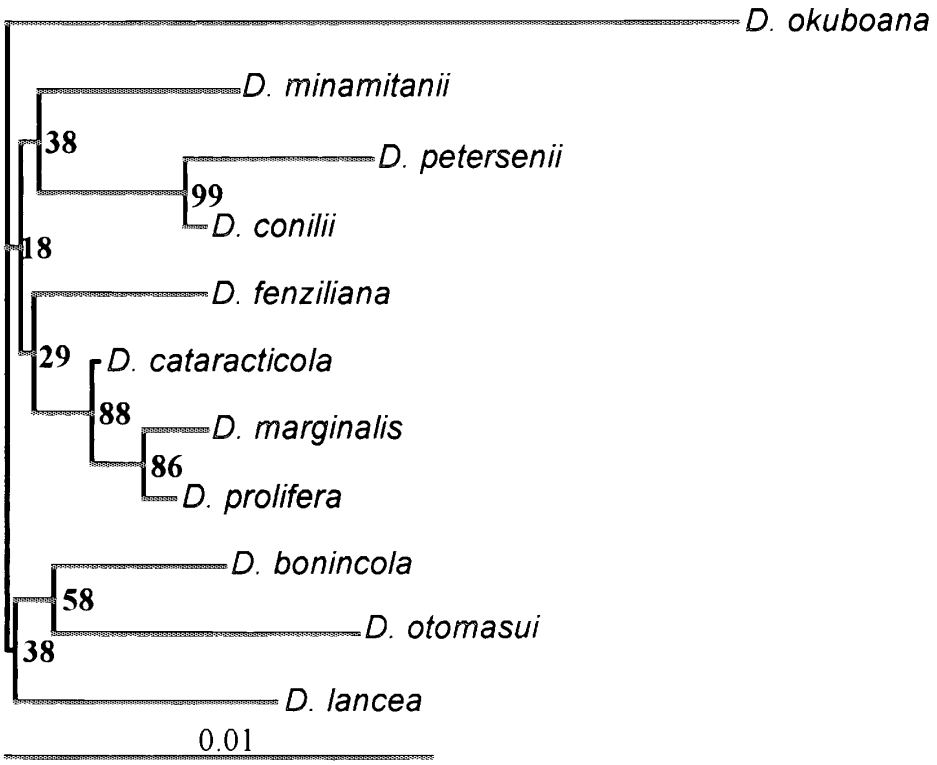

FIG. 3. Tree obtained using neighbor joining (NJ) method. Numbers to right of branches are bootstrap values of 1000 replications. *Deparia okuboana* is an outgroup.

(indigenous; sect. *Deparia*).

2. Leaves slightly narrowed to base, non-gemmiferous; scales dark brown or black; sori median .....*D. fenzliana*
2. Leaves slightly or gradually narrowed to base, gemmiferous; scales brown; sori medial to marginal.
   3. Leaves >25 cm long excluding petiole; petiole >10 cm long; pinnae sessile, >11 cm long, >1.5 cm wide; in woods or around waterfalls.
      4. Rhizomes short, creeping; leaves bipinnatifid to tripinnatifid; sori medial to marginal or extruding from laminae .....*D. prolifera*
      4. Rhizomes ascending to erect; leaves bipinnatifid; sori medial.
         5. Leaves gradually narrowed to base, with a few gemmae; in woods .....*D. marginalis*
         5. Leaves hardly or slightly narrowed to base; abundantly gemmiferous; in waterfalls .....*D. cataracticola*
   3. Leaves to 26 cm excluding petiole; petiole 5-9 cm long; pinnae short-stalked, to 5 x 1.1 cm; rheophyte .....*D. kaalaana*
1. Rhizomes long creeping; margin of indusia toothed (naturalized; sect. *Athyriopsis*) .....*D. petersenii* var. *petersenii*

Phylogenetic analysis

The morphological comparison failed to unravel the systematic relationship of *Deparia cataracticola* with certainty. From the molecular data (Figs. 3, 4), *Deparia cataracticola* is sister to a clade of *D. marginalis* and *D. prolifera* with a high support (>80%). Unique nucleotide substitutions do not support the possibilities that *D. cataracticola* is an interspecific hybrid of

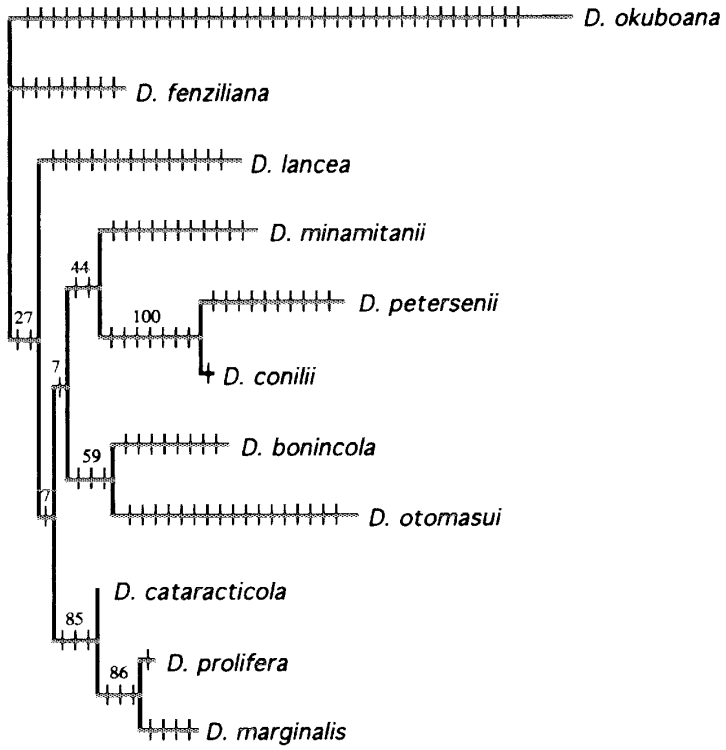

FIG. 4. One of four most parsimonious (MP) trees (length = 135 steps; CI = 0.859; RI = 0.612). Bars on branches are nucleotide substitutions (ACCTRAN optimization); numbers above branches are bootstrap values of 1000 replications. *Deparia okuboana* is outgroup.

congeneric species or an ecotype of the variable species *D. prolifera*. The data also support the previous classification suggesting a close relationship of *D. marginalis* and *D. prolifera* (see Kato 1984). *Deparia bonincola*, an endemic to the Bonin (Ogasawara) Islands, which was included in sect. *Deparia* by Kato (1984), is sister to *D. otomasui* of sect. *Athyriopsis*, with a low bootstrap value (58%), suggesting that sect. *Deparia* sensu Kato may not be monophyletic. The present analysis could not determine the systematic position of *D. fenzliana*, which is morphologically more similar to the Hawaiian *Deparia* than to any other congeneric species (Kato 1984). These possibilities should be investigated by further study. Sano *et al.* (2000a, b) also did not resolve the infrageneric relationships of *Deparia* well.

Deparia cataracticola is morphologically and systematically distinct from the naturalized *D. petersenii* (sect. *Athyriopsis*), as noted above. The remote relationship is supported by molecular data showing that the clade of *D. cataracticola*, *D. marginalis*, and *D. prolifera* is separated from the clade of *D. petersenii* and *D. conilii* (Figs. 3, 4; for comparison see Sano *et al.* 2000a, Figs. 2, 3).

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